

Application No. 09/626,566
 Filed: July 27, 2000
 Group Art Unit: 1651
 Confirmation No.: 9704

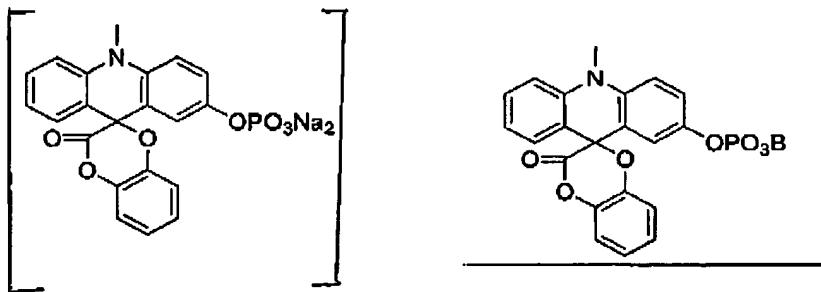
AMENDMENT TO THE CLAIMS

1-7. (Cancelled)

8. (Currently amended) The chemiluminescent substrate of claim 43 wherein said counter ions A are ion A⁻ is selected from the group consisting of CH₃SO₄⁻, FSO₃⁻, CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻, and NO₃⁻.

9-21. (Cancelled)

22. (Currently amended) The chemiluminescent substrate of claim 61 having the following structure:



23-24. (Cancelled)

25. (Cancelled)

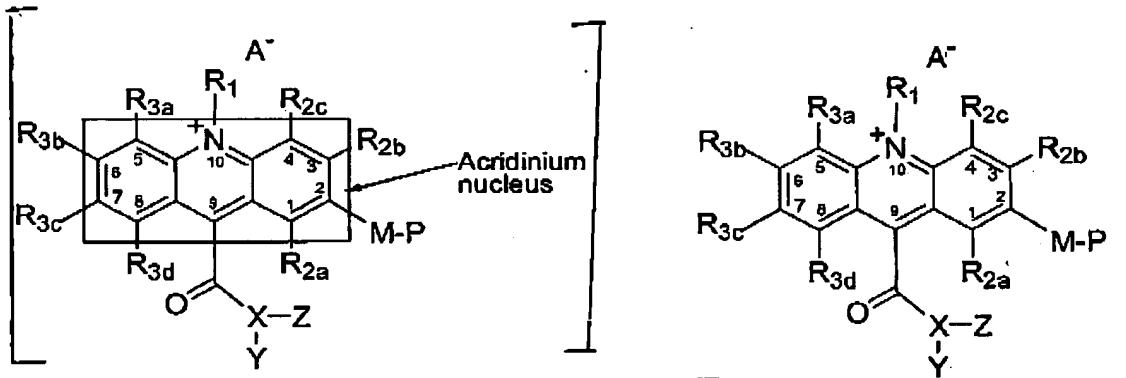
26-28. (Cancelled)

29. (Cancelled)

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30-42. (Cancelled)

43. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO_3Na_2 or PO_3B or a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and Mg ;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl and sulfobutyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} are hydrogen;

A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group

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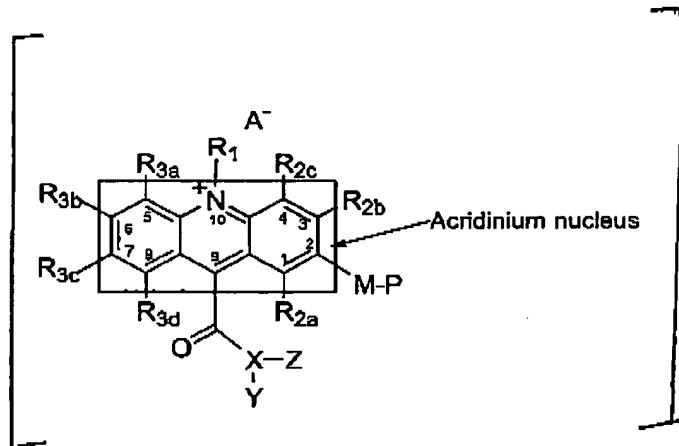
that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

44. (Currently amended) A-The chemiluminescent substrate of claim 43, wherein a hydrolytic enzyme, said substrate having the structure,



wherein

P is PO₃B PO₃Na₂ or a sugar moiety;

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M is oxygen;

R₄ is selected from the group consisting of methyl,
sulforpropyl and sulfobutyl;

R_{2a}, R_{2b}, R_{2c}, R_{3a}, R_{3b}, R_{3c} and R_{3d}, are hydrogen;

A⁻ is a counter ion for the electroneutrality of the
quaternary nitrogen of the acridinium compounds, said A⁻ not being
present if said R₄ substituent contains a strongly ionizable group
that can form an anion and pair with the quaternary ammonium
cationic moiety; and

X is O; Y is selected from the group consisting of phenyl,
 (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-
 4'-carboxyl)phenyl; and Z is omitted.

45. (Currently amended) The chemiluminescent substrate of claim
 43, wherein

P is PO₃Na₂PO₃B;

X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

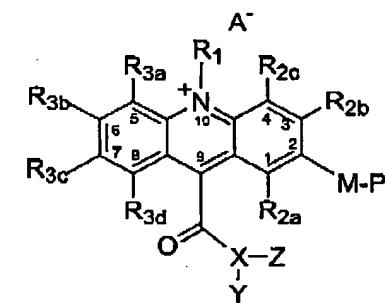
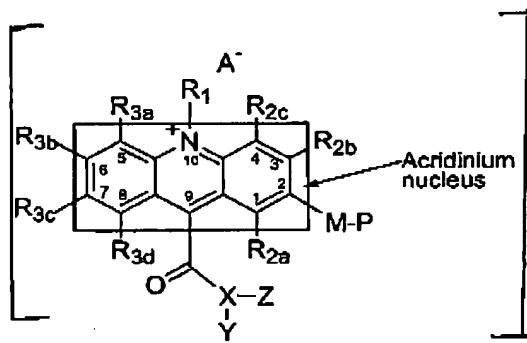
46. (Currently amended) The chemiluminescent substrate of claim
 43, wherein

P is PO₃Na₂ PO₃B;

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X is S; Y is selected from the group consisting of phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, and (2',6'-dimethyl-4'-carboxyl)phenyl; and Z is omitted.

47. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO3Na2—PO3B or a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na2, H2, K2, Ca and Mg;

M is oxygen;

R1 is selected from the group consisting of methyl, sulfoalkyl and carboxymethyl;

R2a, R2b, R2c, R3a, R3b, R3c and R3d can be the same or different, and are selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

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A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O , N ~~or~~ and S , such that,

when X is O or S , Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N , Z is toluenesulfonyl, and Y is carboxypropyl.

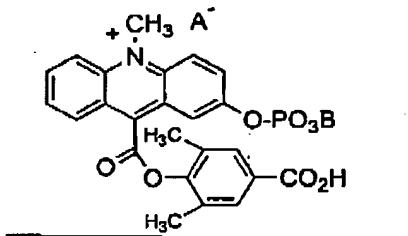
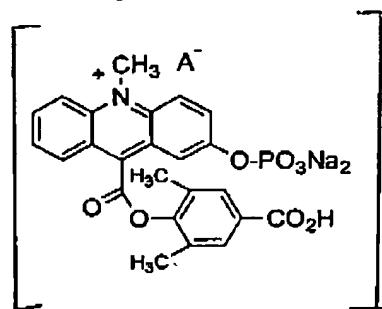
48. (Currently amended) The chemiluminescent substrate of claim 47 wherein said counter ~~ions~~ ion A^- ~~are~~ is selected from the group consisting of $CH_3SO_4^-$, FSO_3^- , $CF_3SO_3^-$, $C_4F_9SO_3^-$, $CH_3C_6H_4SO_3^-$, halide, CF_3COO^- , CH_3COO^- , and NO_3^- .

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49. (Cancelled)

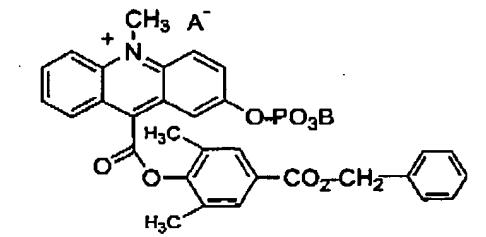
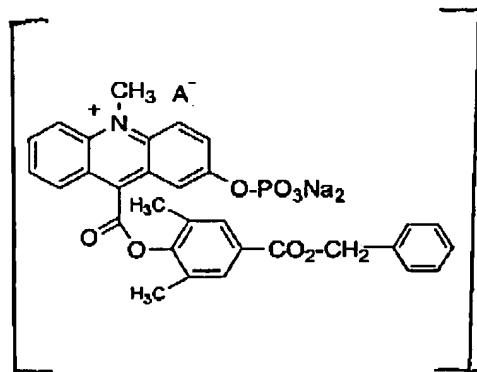
50. (Cancelled)

51. (Currently amended) The chemiluminescent substrate of Claim
claim 43 -having the structure,



wherein A⁻ is selected from the group consisting of CH₃SO₄⁻, FSO₃⁻, CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻, and NO₃⁻.

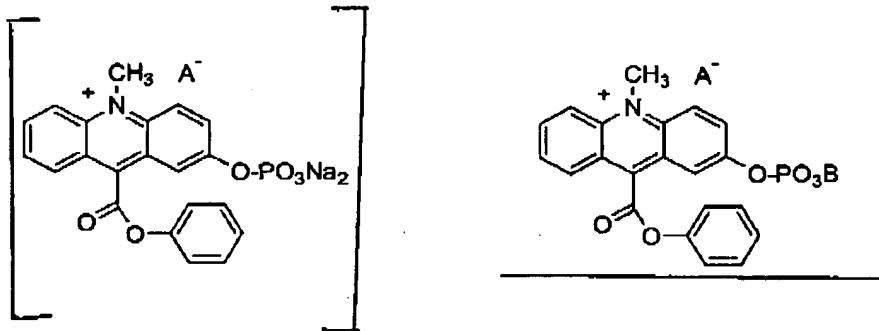
52. (Currently amended) The chemiluminescent substrate of Claim
claim 43 having the structure,



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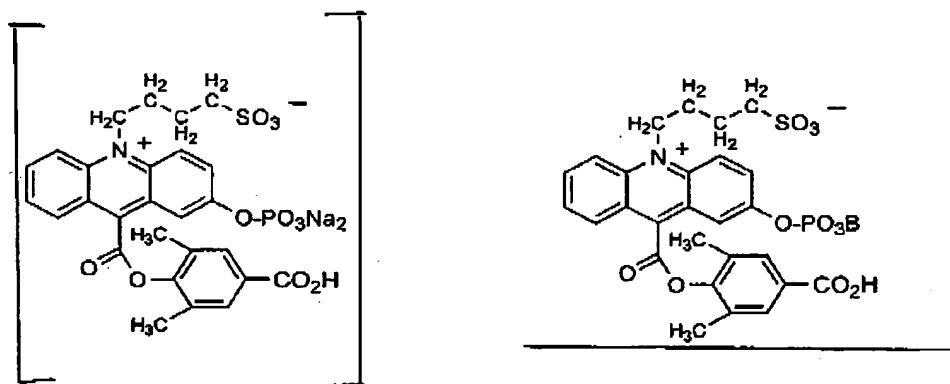
wherein A^- is selected from the group consisting of $CH_3SO_4^-$, FSO_3^- , $CF_3SO_3^-$, $C_4F_9SO_3^-$, $CH_3C_6H_4SO_3^-$, halide, CF_3COO^- , CH_3COO^- , and NO_3^- .

53. (Currently amended) The chemiluminescent substrate of Claim claim 43 having the structure,



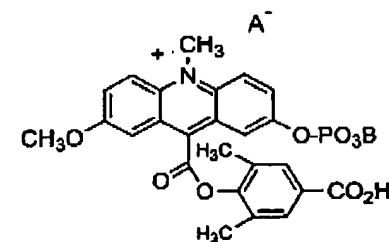
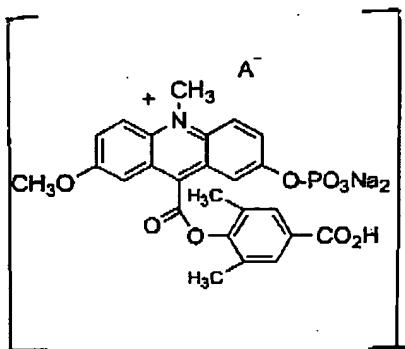
wherein A^- is selected from the group consisting of $CH_3SO_4^-$, FSO_3^- , $CF_3SO_3^-$, $C_4F_9SO_3^-$, $CH_3C_6H_4SO_3^-$, halide, CF_3COO^- , CH_3COO^- and NO_3^- .

54. (Currently amended) The chemiluminescent substrate of Claim claim 43 having the structure



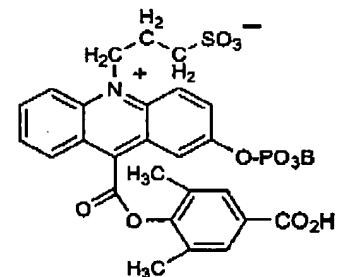
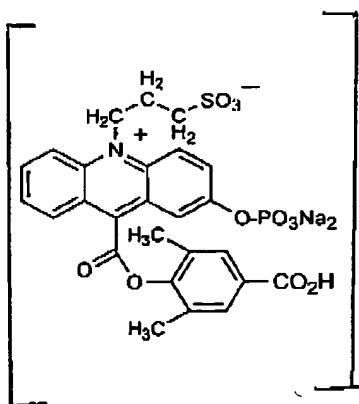
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55. (Currently amended) The chemiluminescent substrate of Claim
claim 47 having the structure,



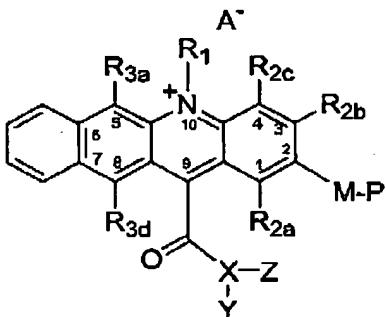
wherein A⁻ is selected from the group consisting of CH₃SO₄⁻, FSO₃⁻, CF₃SO₃⁻, C₄F₉SO₃⁻, CH₃C₆H₄SO₃⁻, halide, CF₃COO⁻, CH₃COO⁻ and NO₃⁻.

56. (Currently amended) The chemiluminescent substrate of Claim
claim 43 having the structure



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57. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO₃B or selected from the group consisting of PO₃H₂, PO₃K₂, PO₃(NH₄)₂, PO₃Ca, PO₃Mg, PO₃Na₂, a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na₂, H₂, K₂, Ca and MgC(=O)R group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R₁ is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl; R_{2a}, R_{2b}, R_{2c}, R_{3a}, and R_{3d} can be the same or different, and are selected from the group consisting of hydrogen, methyl, methoxy, halides, and cyano (-CN);

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A^- is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A^- not being present if said R_1 substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

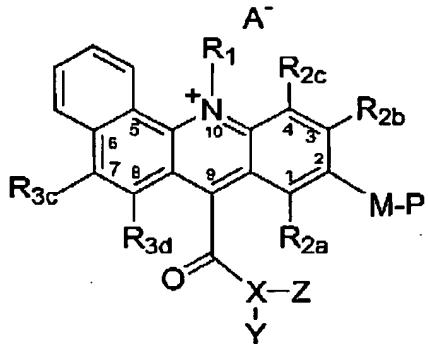
X is selected from the group consisting of O , N or S , such that,

when X is O or S , Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N , Z is toluenesulfonyl, and Y is carboxypropyl.

58. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure

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wherein

P is PO_3B or selected from the group consisting of PO_3H_2 ,
 PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and B is a
divalent cation or two monovalent cations selected from the group
consisting of Na_2 , H_2 , K_2 , Ca and $\text{MgC}(-\text{O})\text{R}$ group wherein R is an
alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R₁ is selected from the group consisting of methyl,
sulforpropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a}, R_{2b}, R_{2c}, R_{3c} and R_{3d} can be the same or different, and
are selected from a—the group consisting of hydrogen, methyl,
methoxy, halides, and cyano ($-\text{CN}$)—;

A⁻ is a counter ion for the electroneutrality of the
 quaternary nitrogen of the acridinium compounds, said A⁻ not being
 present if said R₁ substituent contains a strongly ionizable group

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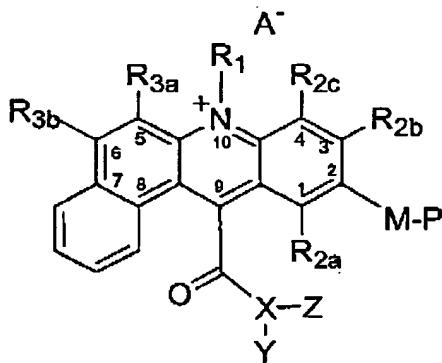
that can form an anion and pair with the quaternary ammonium cationic moiety; and

X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

59. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



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wherein

P is PO₃B or selected from the group consisting of PO₃H₂, PO₃K₂, PO₃(NH₄)₂, PO₃Ca, PO₃Mg, PO₃Na₂, a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na₂, H₂, K₂, Ca and Mg(-O)R group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R₁ is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a}, R_{2b}, R_{2c}, R_{3a} and R_{3b} can be the same or different, and are selected from the group consisting of hydrogen, methyl, methoxy, -halides, and cyano (-CN);

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

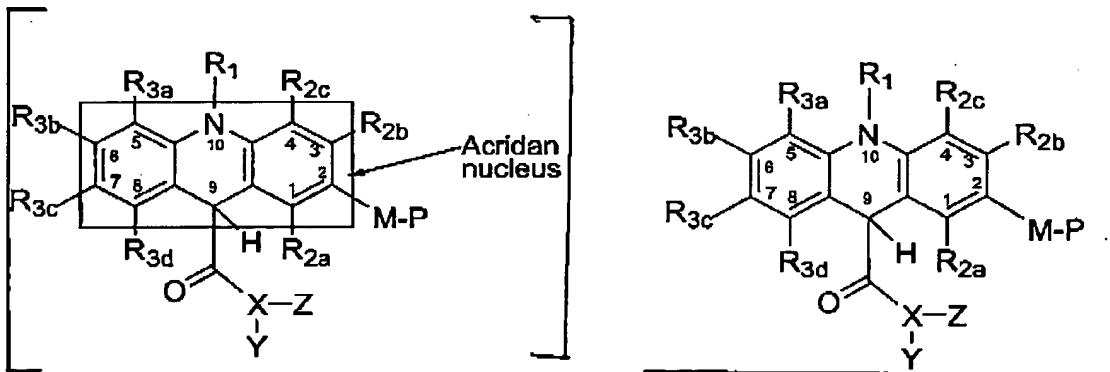
X is selected from the group consisting of O, N or S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-

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dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

60. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO_3B or selected from the group consisting of PO_3H_2 , PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and $\text{MgG}(-\text{O})\text{R}$ group wherein R is an alkyl group having 1 to 6 carbon atoms;

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M is oxygen;

R₁ is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a}, R_{2b}, R_{2c}, R_{3a}, R_{3b}, R_{3c} and R_{3d} can be the same or different, and are selected from a the group consisting of hydrogen, methyl, -methoxy, halides, and cyano (-CN);

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

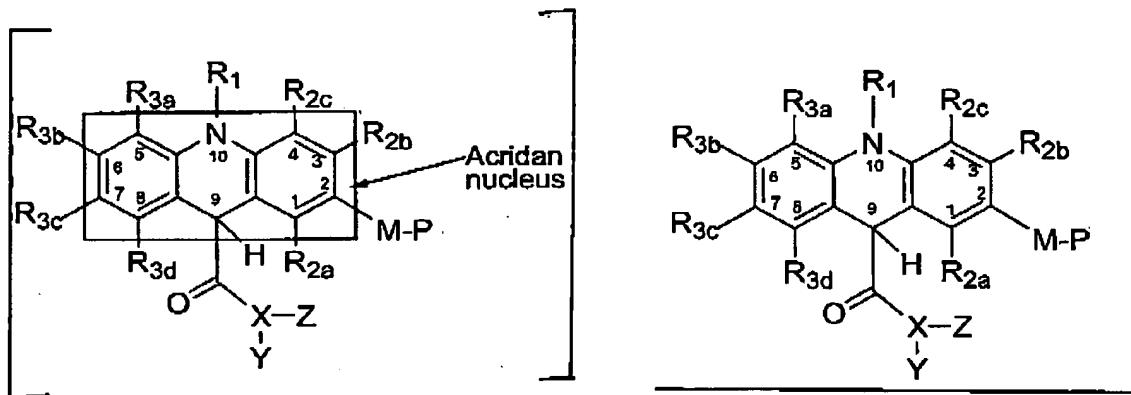
X is selected from the group consisting of O, N or and S, such that,

when X is O or S, Y is selected from the group consisting of phenyl, (2'-methyl)phenyl, (2'-methoxy)phenyl, (2',6'-dimethyl)phenyl, (2'-methyl-6'-methoxy)phenyl, (2',6'-dimethyl-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethoxy-4'-benzyloxycarbonyl)phenyl, (2'-methyl-6'-methoxy-4'-benzyloxycarbonyl)phenyl, (2',6'-dimethyl-4'-carboxyl)phenyl, (2',6'-dimethoxy-4'-carboxyl)phenyl, and (2'-methyl-6'-methoxy-4'-carboxyl)phenyl; and Z is omitted; and

when X is N, Z is toluenesulfonyl, and Y is carboxypropyl.

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61. (Currently amended) A chemiluminescent substrate of a hydrolytic enzyme, said substrate having the structure



wherein

P is PO_3B or selected from the group consisting of PO_3H_2 ,
 PO_3K_2 , $\text{PO}_3(\text{NH}_4)_2$, PO_3Ca , PO_3Mg , PO_3Na_2 , a sugar moiety and B is a divalent cation or two monovalent cations selected from the group consisting of Na_2 , H_2 , K_2 , Ca and $\text{MgC}(-\text{O})\text{R}$ group wherein R is an alkyl group having 1 to 6 carbon atoms;

M is oxygen;

R_1 is selected from the group consisting of methyl, sulfopropyl, sulfobutyl, sulfoalkyl, and carboxymethyl;

R_{2a} , R_{2b} , R_{2c} , R_{3a} , R_{3b} , R_{3c} and R_{3d} can be the same or different, and are selected from a the group consisting of hydrogen, methyl, methoxy, halides, and cyano $(-\text{CN})$;

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R₁₁ is selected from the group consisting of hydrogen, -R,
substituted or unsubstituted aryl, halides, nitro, sulfonate,
sulfate, phosphonate, -CO₂H, -C(O)OR, cyano (-CN), -SCN, -OR, -SR,
-SSR, -C(O)R, -C(O)NHR, ethylene glycol and polyethylene glycol,
where R is an alkyl group having 1 to 6 carbon atoms;

A⁻ is a counter ion for the electroneutrality of the quaternary nitrogen of the acridinium compounds, said A⁻ not being present if said R₁ substituent contains a strongly ionizable group that can form an anion and pair with the quaternary ammonium cationic moiety; and

X₁ -and X₂ -are the same or different and are selected from the group consisting of O, N or S, such that,

when at least one of X₁ -and X₂ are is O or S, R₁₁ is selected from the group consisting of hydrogen, -R, substituted or unsubstituted aryl, halides, nitro, sulfonate, sulfate, phosphonate, -CO₂H, -C(O)OR, cyano (-CN), -SCN, -OR, -SR, -SSR, -C(O)R, -C(O)NHR, ethylene glycol, or polyethylene glycol, where R is as defined above, andthe corresponding Z₁ -and or Z₂ are is omitted; and

when at least one of X₁ -and X₂ is N, the corresponding Z₁ and or Z₂ are is hydrogen, alkyl, aryl or toluenesulfonyl, and R₁₁ is carboxypropyl.